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22 March, 1954.

Dear Joshua,

Your long letter reached me when I returned to work today. Thanks for your offer to present any comment I might like to make at the Oak Ridge symposium. I appreciate this, but there is really no point of my own that I want to make . I really have no new significant data which I would be prepared to stand over, though the kinetics work using the T phages which I started at Pasadena has offered me several new lines which I think may be productive. I will tell you briefly my ideas about this first. The last para. of your letter suggests to me that you may already have a considerable inkling of these ideas from the rather meagre clues I gave you! First let me say that I have an open mind on the question of when the eliminations occur and fully appreciate the significance of your recent experiments on the metatypic hets. and the segregation of isolated zygotes. Nevertheless, if you do'nt mind, I would like to keep this important point open since my ideas were born in an attempt to devise an experiment to clear it up. I like to satisfy myself about things in my own way! The fertility of S^sF+ X S^rF- crosses on S-MA suggested either that S^s dominance was not expressed in the transient zygote (as opposed to your hets.) or else that the S^s allele of the F+ parent was not present in the xygote. This was confirmed by the fact that neither T_3 nor T_6 had any effect on zygotes in the system $Hfr.T_3{}^s.T_6{}^s$ X $F-.T_3{}^r.T_6{}^r$. On the assumption that the zygote is complete but that dominance relationships are not phenotypically expressed in it, the situation would be that while the Hfr parent could be destroyed by phage to which it was sensitive, the Hfr genome within the diploid zygotes would be immune until segregation. If, therefore, the parents are mixed in aerated broth for ¼-½ hr. so that zygotes are formed, the Hfr parent then destroyed by phage, the residue treated with anti-phage serum and washed, and then plated, the products of zygote sergegation retrospectively identified by replica plating should show the Hfr parental type in a proportion of colonies. I realise the technical difficulties involved but I think, from my experience with the system, that these can be overcome. Single cell isolation would greatly improve this experiment but I have not yet had any experience with this technique. I also intend to extend this method

I mean, "did not kill [illegible] appreciable proportion". I have not yet scored such recombinants so closely to see if φ induces any selection — see below.

to an investigation of the peculiarities of Lac_1 inheritance. In my Hfr.Lac+ X W677.F- cross,Lac+ is consistently inherited by no more than 20-25% of prototrophs. Without going into details of possible theories to explain this low frequency,I thought it possible that it might be due to "elimination" of the Lac+ locus from a proportion of prototrophs. Lac_1 and V_6 appear to be rather closely linked so that any aberration in the inheritance of Lac due to its situation on the chromosome(rather than due to suppressors &c.) should also involve V_6. This seems to be the case. If,in the system Hfr.Lac+.V_6^S X F-.Lac-.V_6^r,the zygotes are all complete for LacV_6(as I anticipate),then these loci should segregate in the usual ratio after treatment with T_6 followed by its neutralisation as described above. Should this prove not to be the case,then either Lac+V_6^S was not represented in those zygotes saved after T_6 treatment or if from which one might suspect that the dominance of V_6 was in fact expressed in these zygotes in which it appeared. This would in turn suggest that the sparing of zygotes in the T_3 experiments was due to absence of the F+ or Hfr V_3^S alleles from them. All this is a very long shot but I intend to try it. Some time back I was toying with the idea that recombination might be only an extended transduction phenomenon,the fairly regular segregation rations being due the frequency with which bits of chromosome of different length were brought over to the F- parent and "replaced" the homologous F- bit,rather than to crossing-over. I was finding the eliminations,and the anomalies in the TL-V_1-Lac segment(Rothfels) and the unexplained interactions too much to swallow in the face of the similarities of the K-12 system to Salmonella transduction; e the taxonomic relationship,the donor-recipient set-up,the involvement of a transmissible agent in the donor strain,the evidence for "breaks". These phage experiments were evolved with this at the back of my mind,but your recent experiments have shaken me. I would like to tell you more about some nice experiments(not so far successful) to prove fusion by the transferrence of vegetative lambda (after induction) to a /λ2. Non-lysogenic F- but feel I should get on to more definite things.

I was very surprised to hear that Tom Nelson had found no substantial difference in behaviour between our two Hfr strains. There is something funny here. I may be fond of speculation but I am a rather careful and self-critical technician and tend to be cautious about my statements of fact. I have checked and rechecked my Hfr strain and am now quite certain of the facts set forth in my CSH paper about it. In addition to these I have examined a series of 300 prototrophs for Mal(total 600),Xyl,Mtl,Ara and found the Hfr allele absent from all save one. I have told you that the S allele has been completely absent from 500. 300 prototrophs on MA + B_1 were all B_1- although a control cross using the parent F+ strain showed about 8% B_1+. You do not say to what extent addition of B_1 to MA increased the yield in Tom's experiments. I have had a lot of difficulty scoring for B_1 and,to be frank,if I got these results I would doubt my scoring and repeat in an F+ controlled experiment using very small inocula. I find I cannot use replica plating for B_1 scoring. I was particularly struck by

the difference between the two strains in connection with the fool-proof(!) marker S. Cavalli, in the MGB and in a typescript of his Rome talk, says that his strain shows Hfr behaviour when S is the selective marker. This is very definitely not the case with mine. Moreover, recently I have tested my strain in recombination with an argininеless, tryptophaneless F- strain of Coli "C" (British N.C. 123). The Tr locus is very closely linked to (or identical with) the V_1^r locus in this strain. The Hfr X CF- cross shows Hfr behaviour on MA + Arg (when selection is made for the Hfr locus Tr+ which probably lies on the TL→Lac "chromosome") but Nfr behaviour on MA + Tr. On the other hand, approximately the same number of prototrophs arise in the equivalent F+ X CF- crosses. I honestly feel there is an important difference between our strains. Both are derivatives of 58-161, so that a comparative test of F+ and the two Hfr strains in crosses with W677, on MA + B_1, scoring for S, Lac, and the other sugars by replica plating should be decisive. Perhaps you and Tom Nelson would consider this. If you do not find a decisive difference, then there must be some peculiar selective difference between our minimal medium or else my strain has changed; I could send you a checked single colony isolation of my strain for recheck. I am sure that neither you nor Tom Nelson will mind my suggesting that the results of scoring for B_1 may be unduly high. I found that when inocula were large, considerable growth of B_1- prototrophs arose on my MA and that the reversion rate to B_1+ tended to be considerable. I therefore went to the trouble of making a dilute suspension of the purified prototroph in saline and transferring from this to the MA with a straight wire. Anyway I would appreciate it if you would check on your results, though there is no hurry about this from my point of view.

As regards the kinetics experiments, I was confused by the fact that Marguerite Vogt had found (unpublished) that mixing F+ and F- strains in buffered saline lead to a large increase in the number of recombinants, although a nutritional supplement was thereafter required for maximal recombination. She was following up Nelson's original work and you have probably seen the typescript. I failed to substantiate the occurrence of any increase in saline with either F+ or Hfr. I will compare the rate of zygote formation in saline, minimal medium and broth using my phage technique.

I did not make a copy of my last letter to you and cannot remember just what I said. I do not remember mentioning any experiments on the separation of F from the cells. I did'nt even attempt this in the short time I had at Caltech. The only relevant thing I have heard is that F+ cells broken up by HF sound appear to have yielded prototrophs, though this has not yet been properly confirmed. This is being done at Urbana. I think that the question of the nature of F is perhaps the most interesting aspect of K-12 genetics and would like to get on to it. As regards my experiment No.1, the term "reciprocal segregant" connoted only those Hfr markers which appear in recombinants formed at high frequency. I fully agree that if your recent work really implies what it appears to imply, and is applicable to my Hfr strain, then the idea of a deficient contribution to the zygote from the Hfr parent is no longer tenable. But I would

like to hold on to this idea for a little longer.

It will probably be some time before I settle down to experimental work again as I have a lot of teaching and routine work ahead of me. However, as soon as I have any worthwhile results I will let you know. I would much appreciate a typescript of your Oak Ridge talk should you have one available. Incidentally, in the report you sent me, it was not clear whether you had found more than one recombinant type segregating from the zygote.

Please give my kind regards to Nelson and Morse if he is still with you. With every good wish to Esther and yourself,

Yours sincerely,

Bill Hayes